Molecular Dynamics Simulation of Damaged DNA's and Repair Enzymes.

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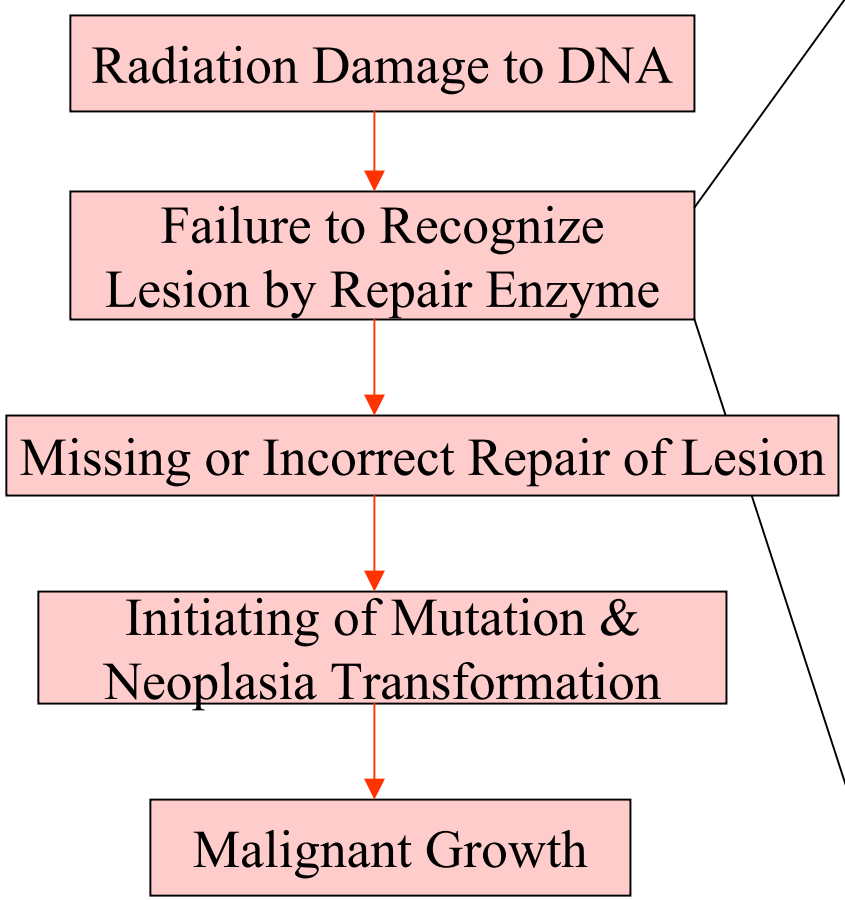
Ionizing radiation and oxidative products of metabolism damage DNA and cause mutations and chromosomal aberrations in cells and organisms.

In response to DNA damages the cells elaborated repair processes – processes that restore the correct DNA sequence and structure.

Recognition process is first step of repair during which repair enzyme recognizes lesion on DNA and forms stabile DNA-enzyme complex.

The pathway of Radiation Carcinogenesis And the Role of Study on Lesion Recognition



- What forces drive repair enzyme toward DNA to recognize lesion?
- Which factors are key ones for proper recognition?
- Why lesion is not recognized?
- Are there specific features of radiation-originated lesion discriminating it from oxidative endogenous damage?

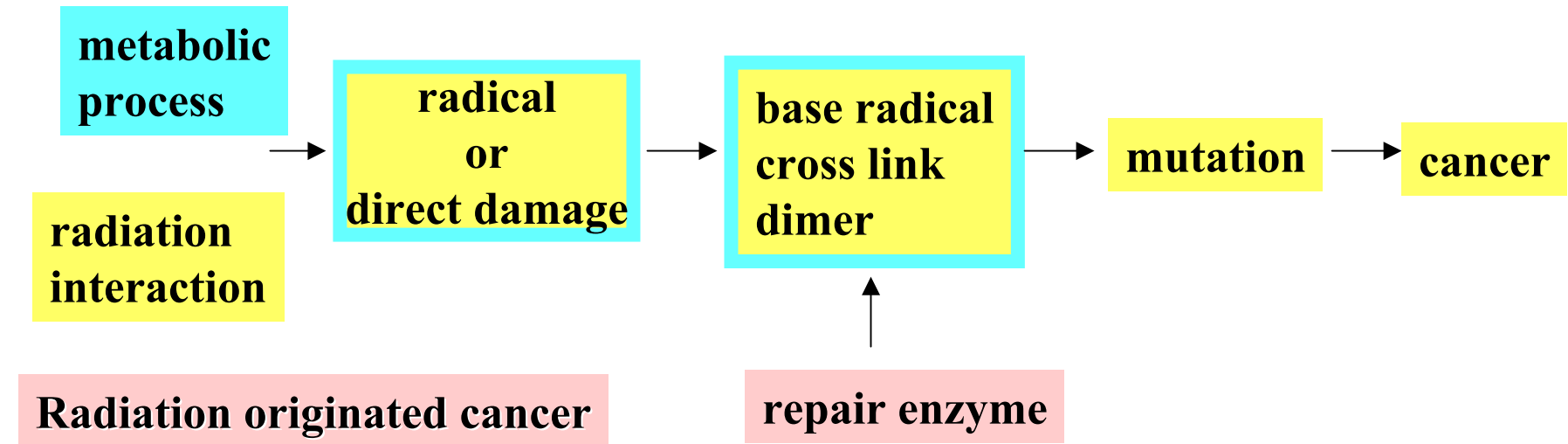
Radiation Risk and Molecular Dynamics Studies on DNA

Risk of radiation-induced cancer is expressed in terms of K-values: K-value is defined as the fractional increase in spontaneous cancer death-rate per centi-sievert of whole-body internal organ-dose.

MD role:

- **Is the damage chemically the same ?**
- **Is it recognized by the same enzyme ?**
- **Is it recognized by the same mechanism ?**

Endogenously originated cancer



Radiation originated cancer

Advantage of the MD simulation

The MD simulation of DNA damage recognition is capable to provide the stepwise description of biomolecular reactions at the radiation lesion site through the capability to describe structural, chemical and chemico-physical reactions in time intervals that correspond to real time of formation and breakage of chemical bonds (order of femtoseconds).

The changes developed on DNA within this time frame (if left un-recognized or un-repaired) may determine properties of the cells.

DNA Base Damages

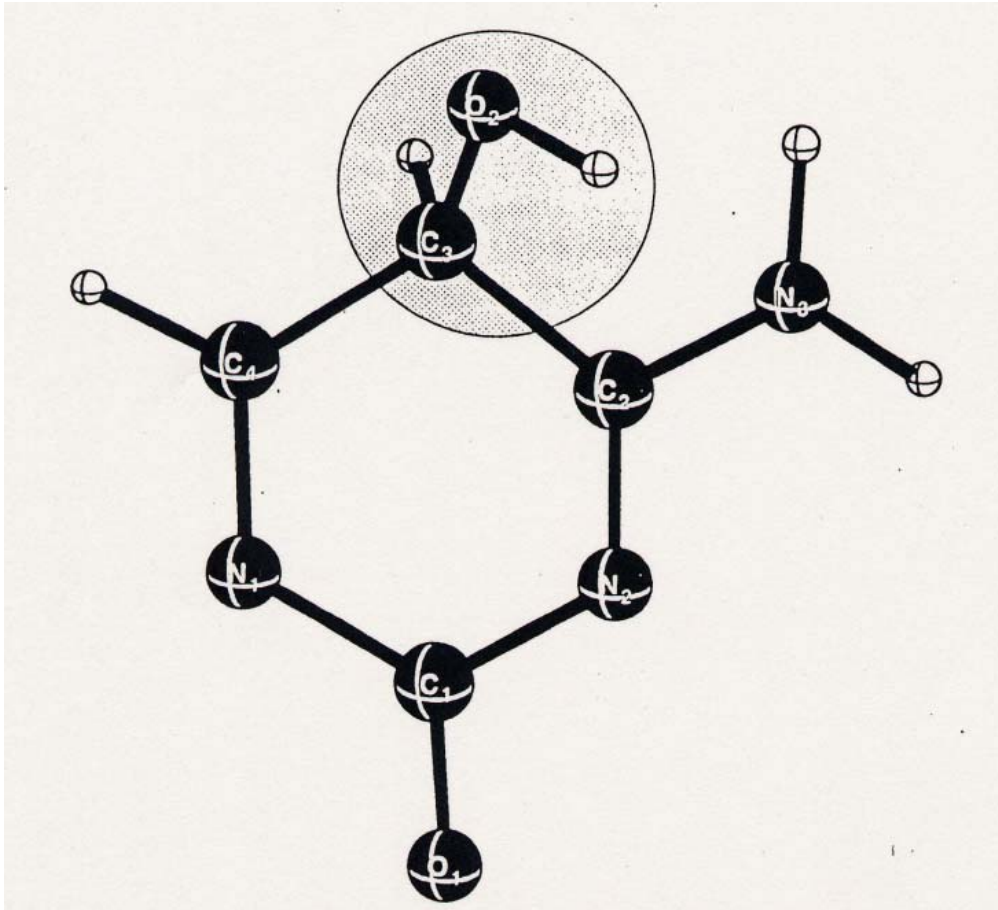
Pyrimidines

1. **Cytosinyl Radical** - study of H-abstraction from the pentose
2. **Thymine Dimer** - product of UV radiation, skin cancer
3. **Thymine Glycol** – oxidative products, Cockayne syndrome

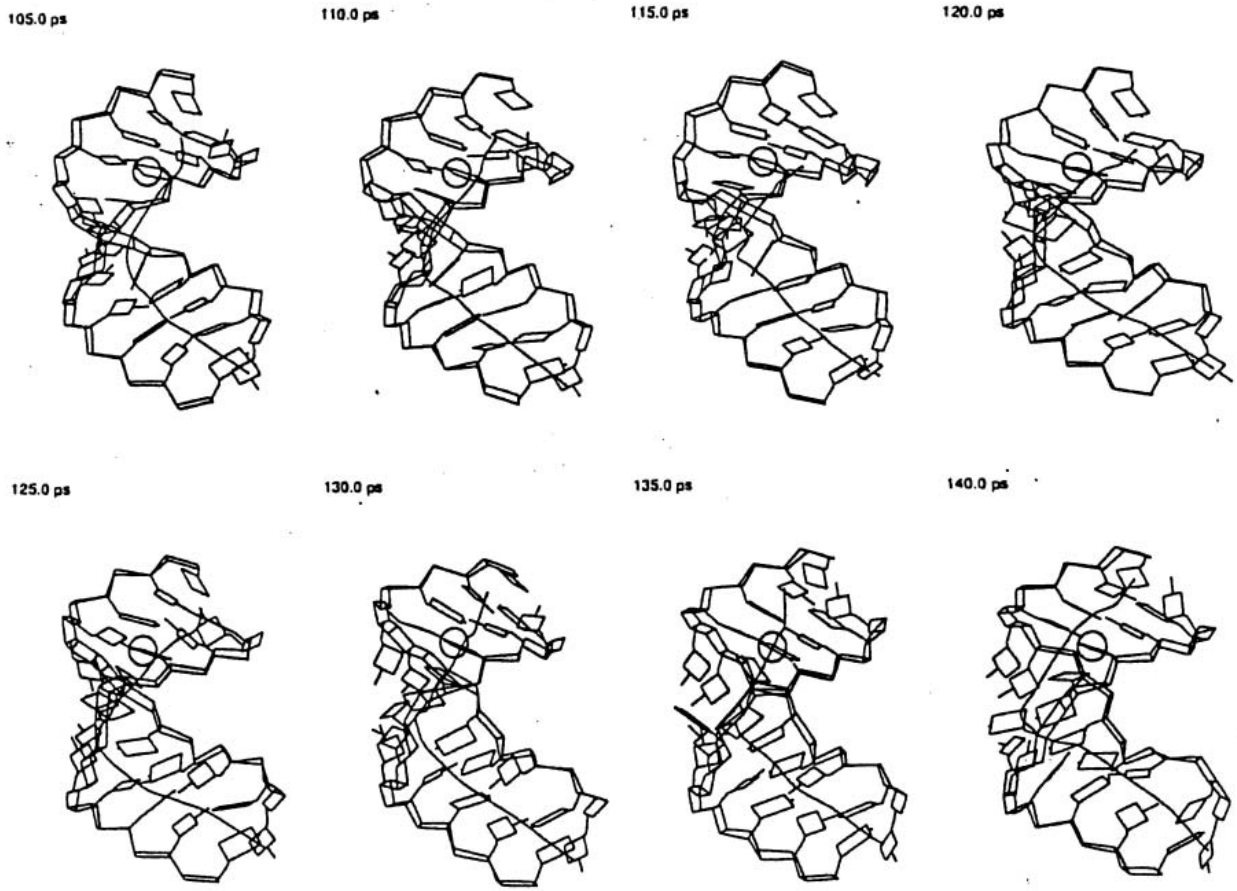
Purines

4. **8-oxoguanine** - endogenous mutagens contributing to transversion mutations (the most common somatic mutation in human cancers)

Cytosinyl radical - study of strand break formation through the intramolecular process of H-abstraction form the pentose and it emphasizes the importance of initial base damage in connection to strand breaks.

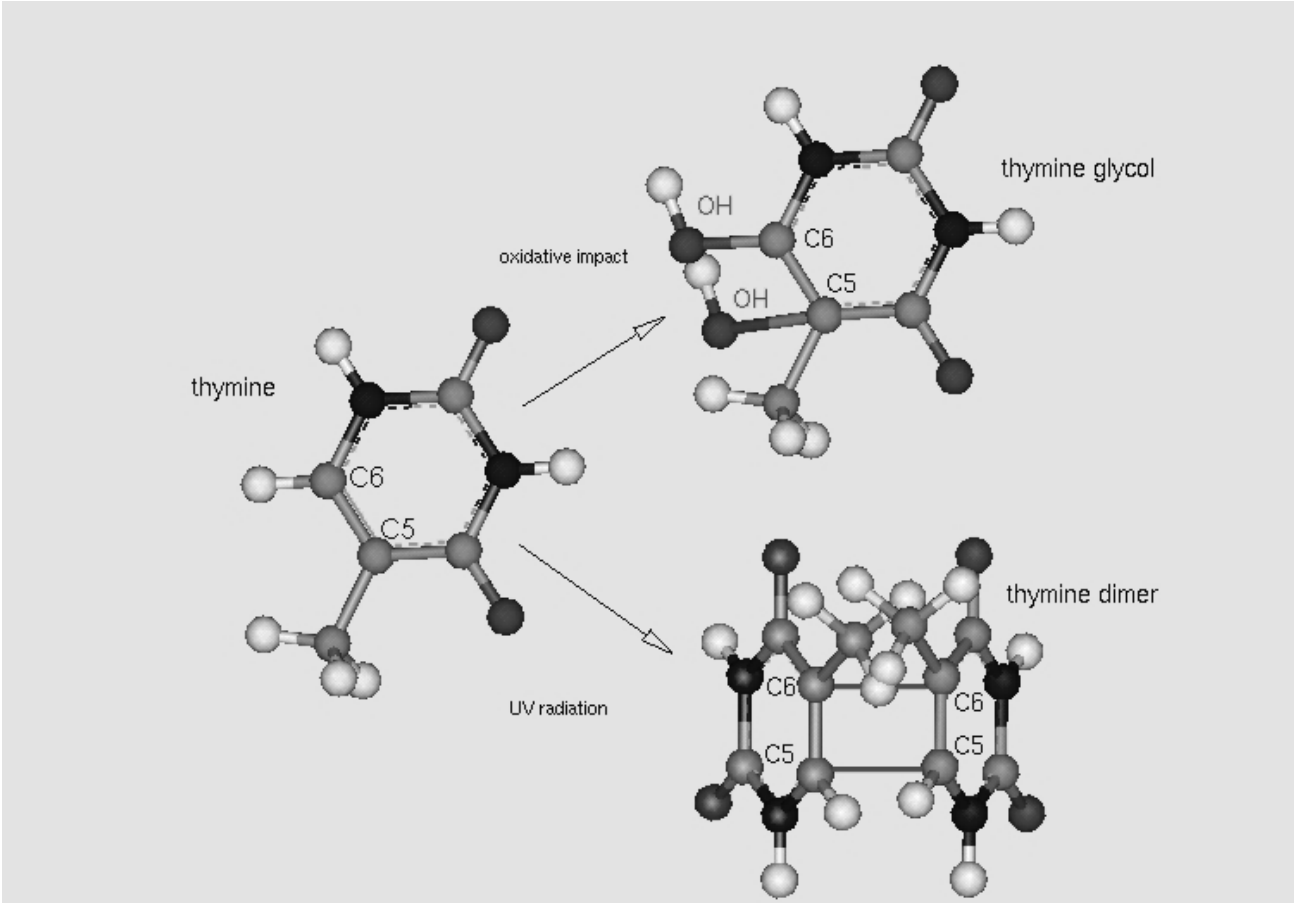


Structure of 5-hydroxy-6-cytosinyl radical (axial position of OH is marked by shadow).



Snapshots of the structures of the **cytosinyl radical** lesioned dodecamer. The cytosinyl radical is encircled.

Thymine dimer (TD) and Thymine Glycol (TG)

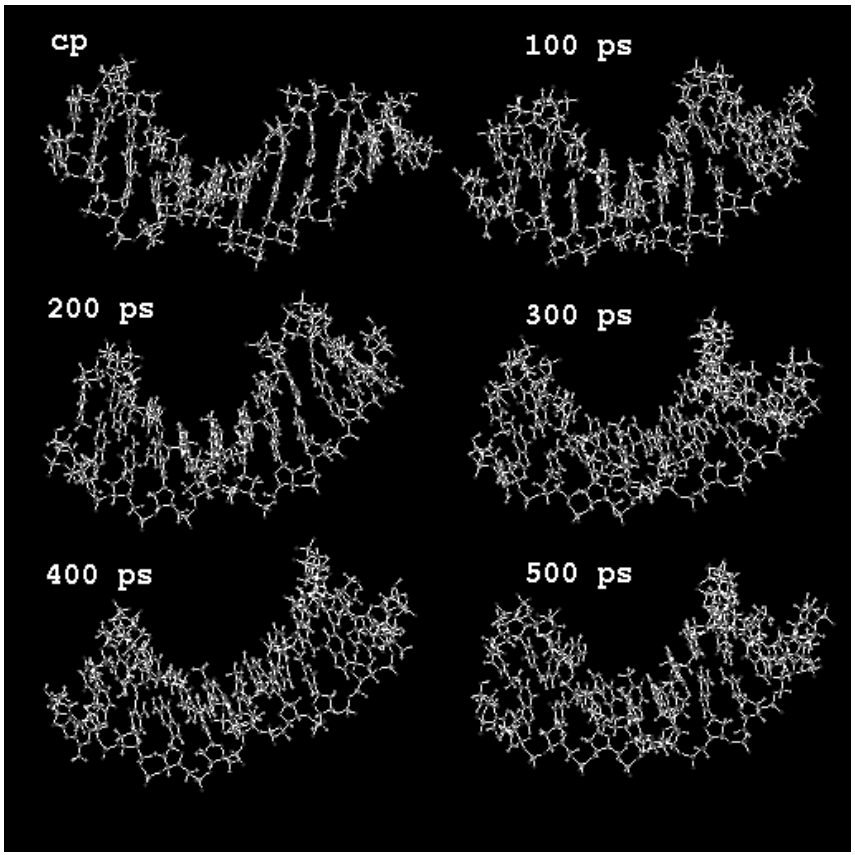


Thymine glycol – oxidative product of radiation or oxidative metabolic processes in cell, causing Cockayne syndrome

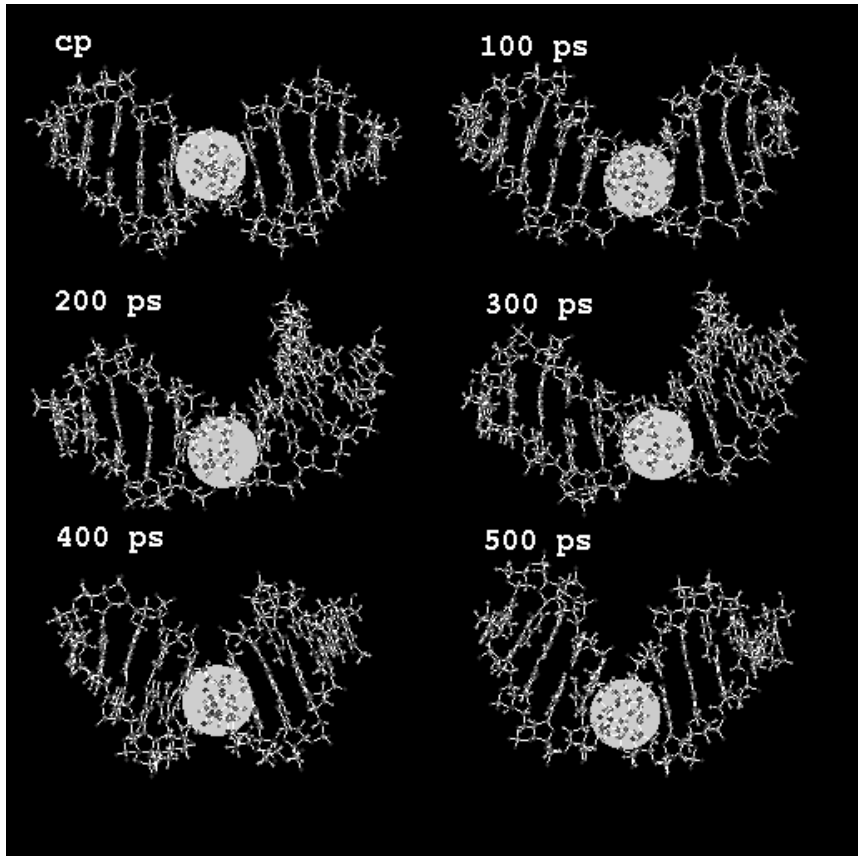
Thymine dimer – product of UV radiation ~ 280 nm, causing skin cancer

Snapshots of TD lesioned DNA molecules

native DNA

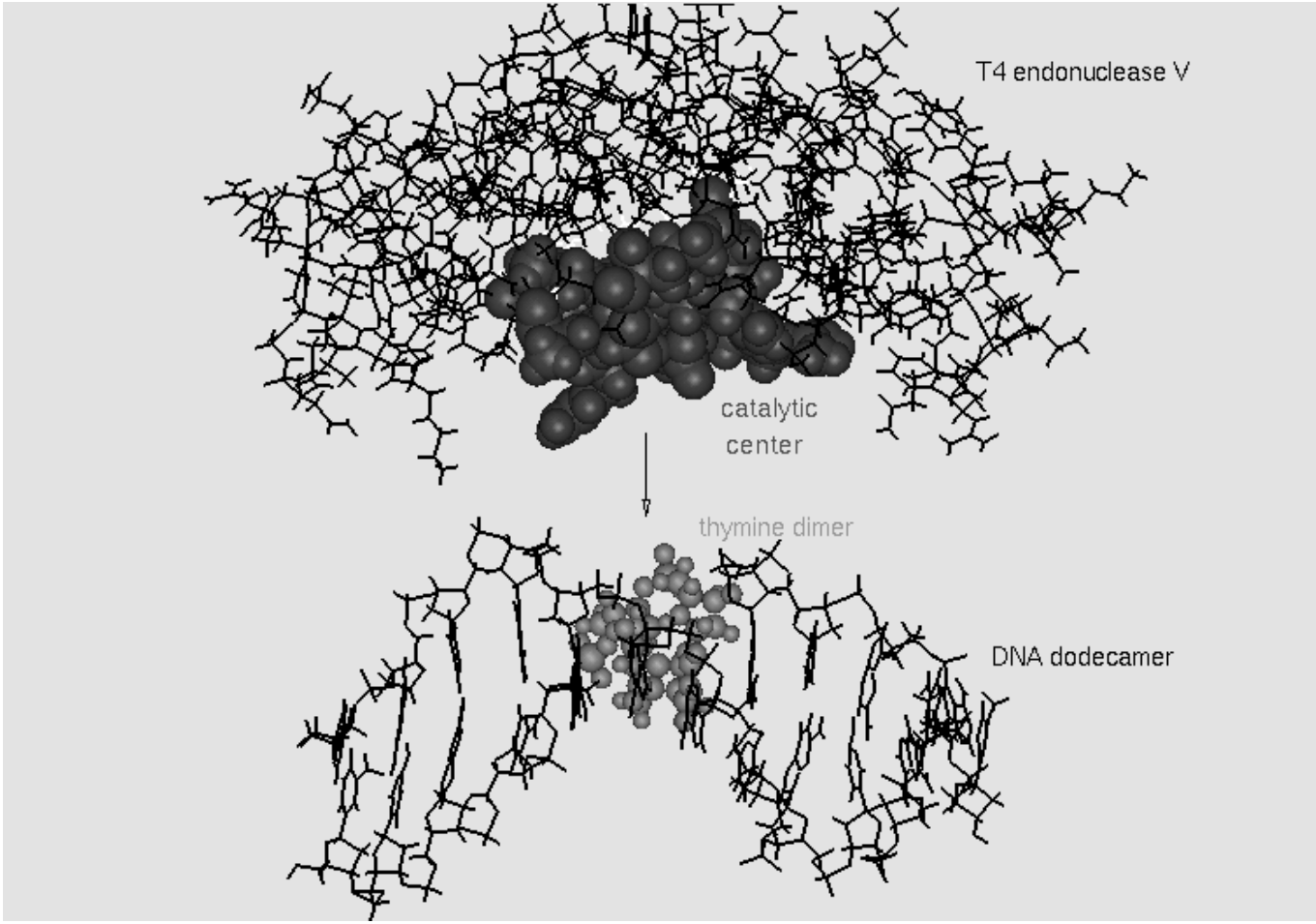


DNA with TD



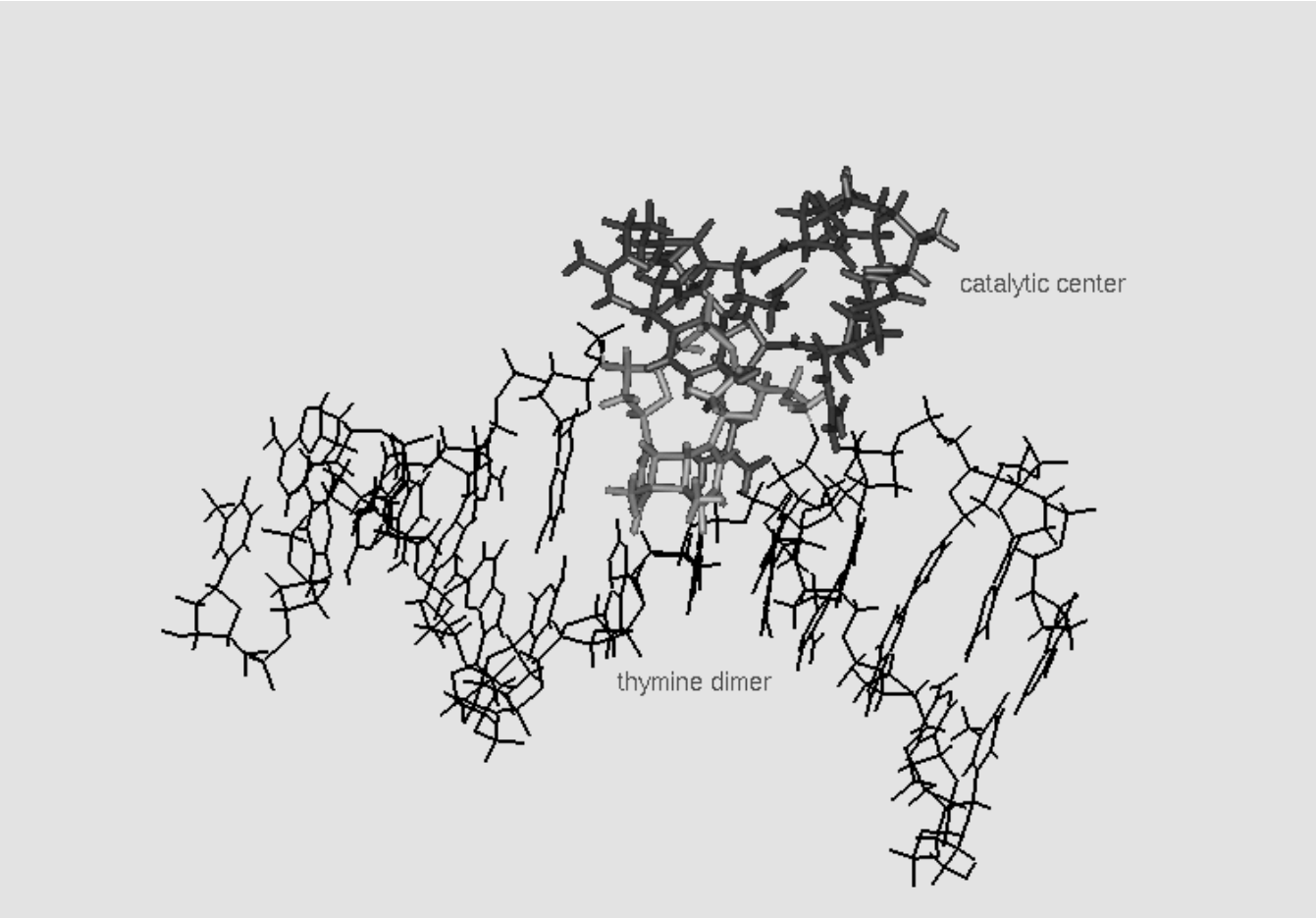
There is observed sharp bending at TD site originated after 200 ps of MD. Structural environment favors docking of T4 endonuclease V onto DNA.

T4 Endonuclease V and TD lesioned DNA dodecamer (position at the beginning of simulation)



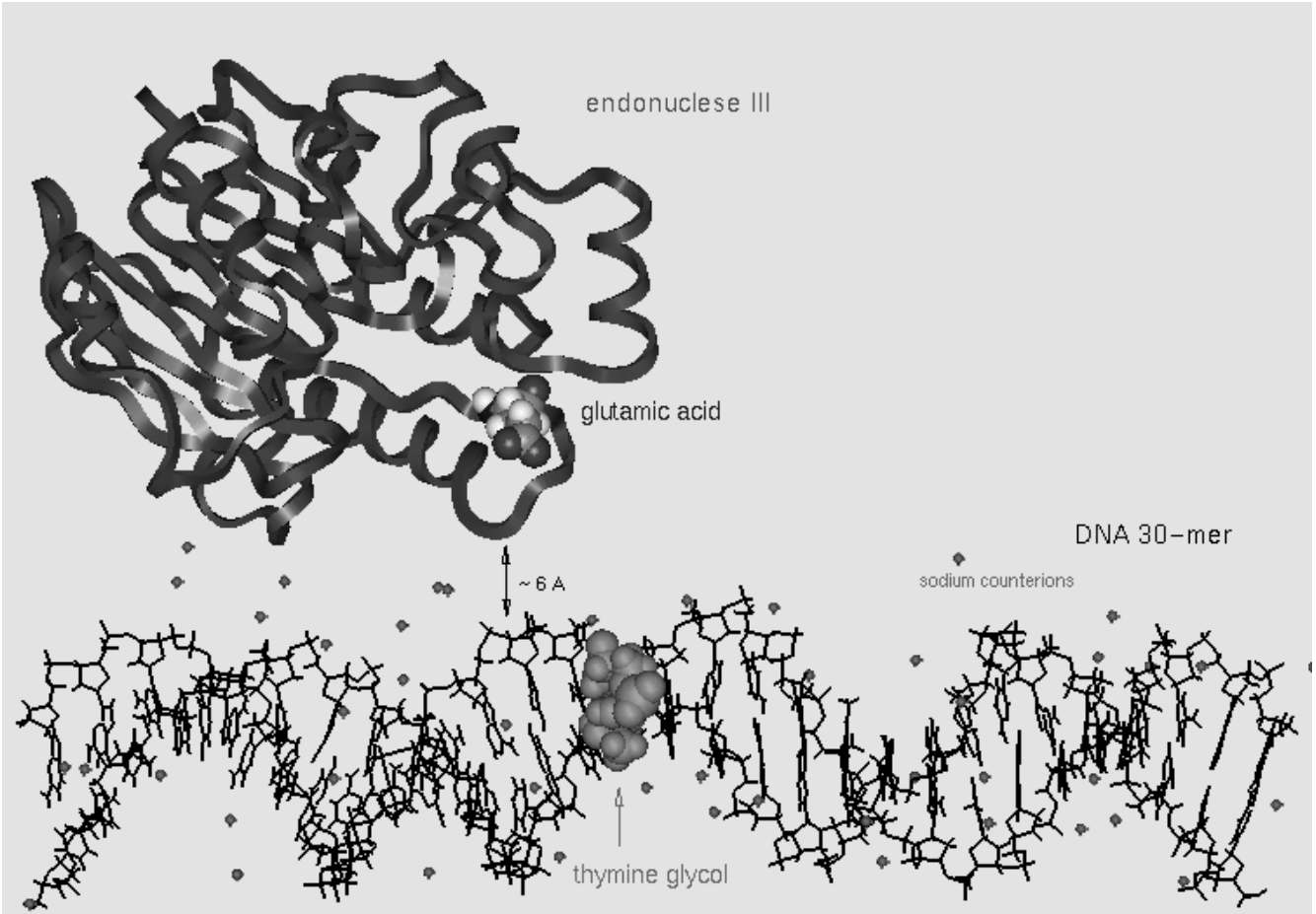
The colored are catalytic center (red) and thymine dimer (green). The relative distance of the closest atoms was about 5Å.

TD lesioned DNA dodecamer complexed with catalytic center of T4 Endonuclease V

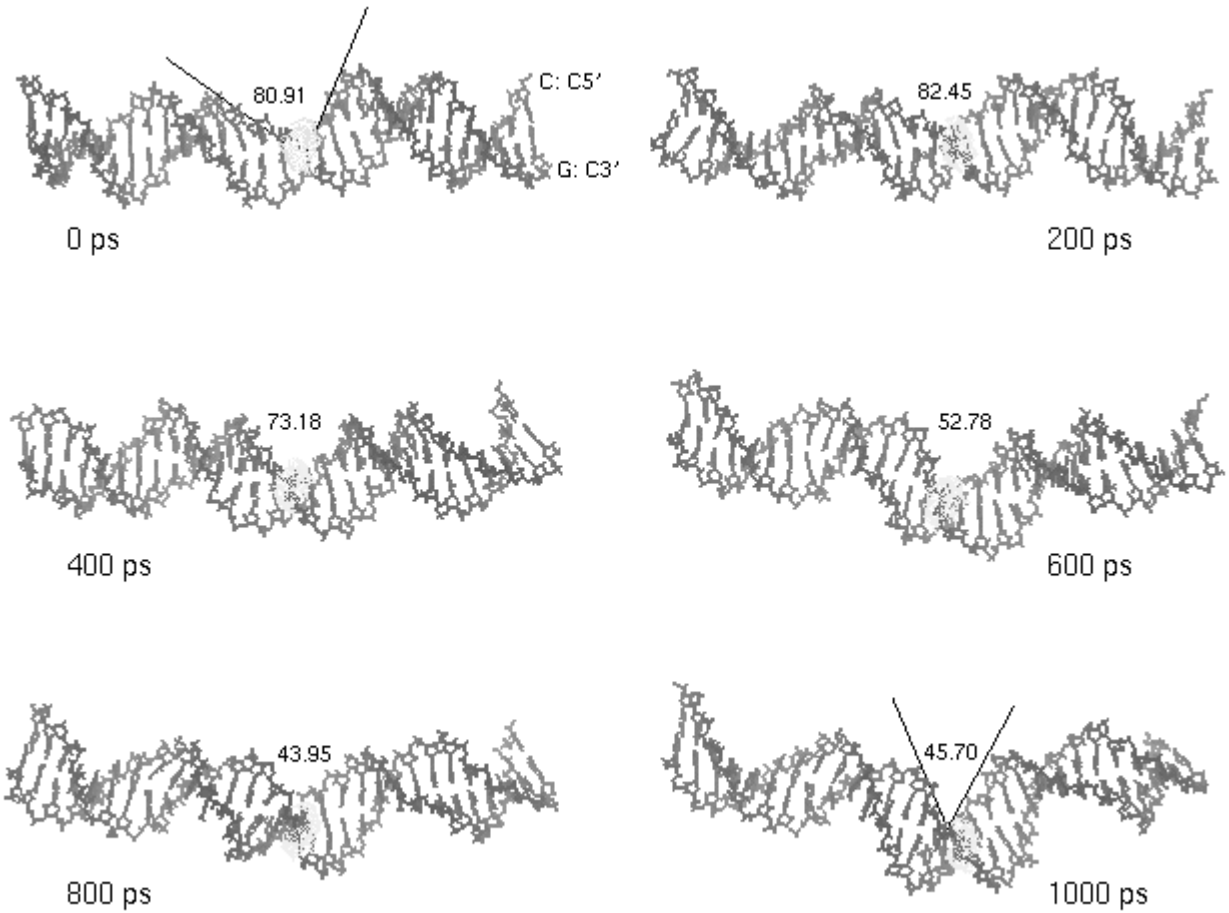


Catalytic center – Arg22, Glu23, Arg26, Thr2 of enzyme formed complex with DNA after 100 ps of MD simulation.

Endonuclease III and TG lesioned DNA 30-mer (position at the beginning of simulation)

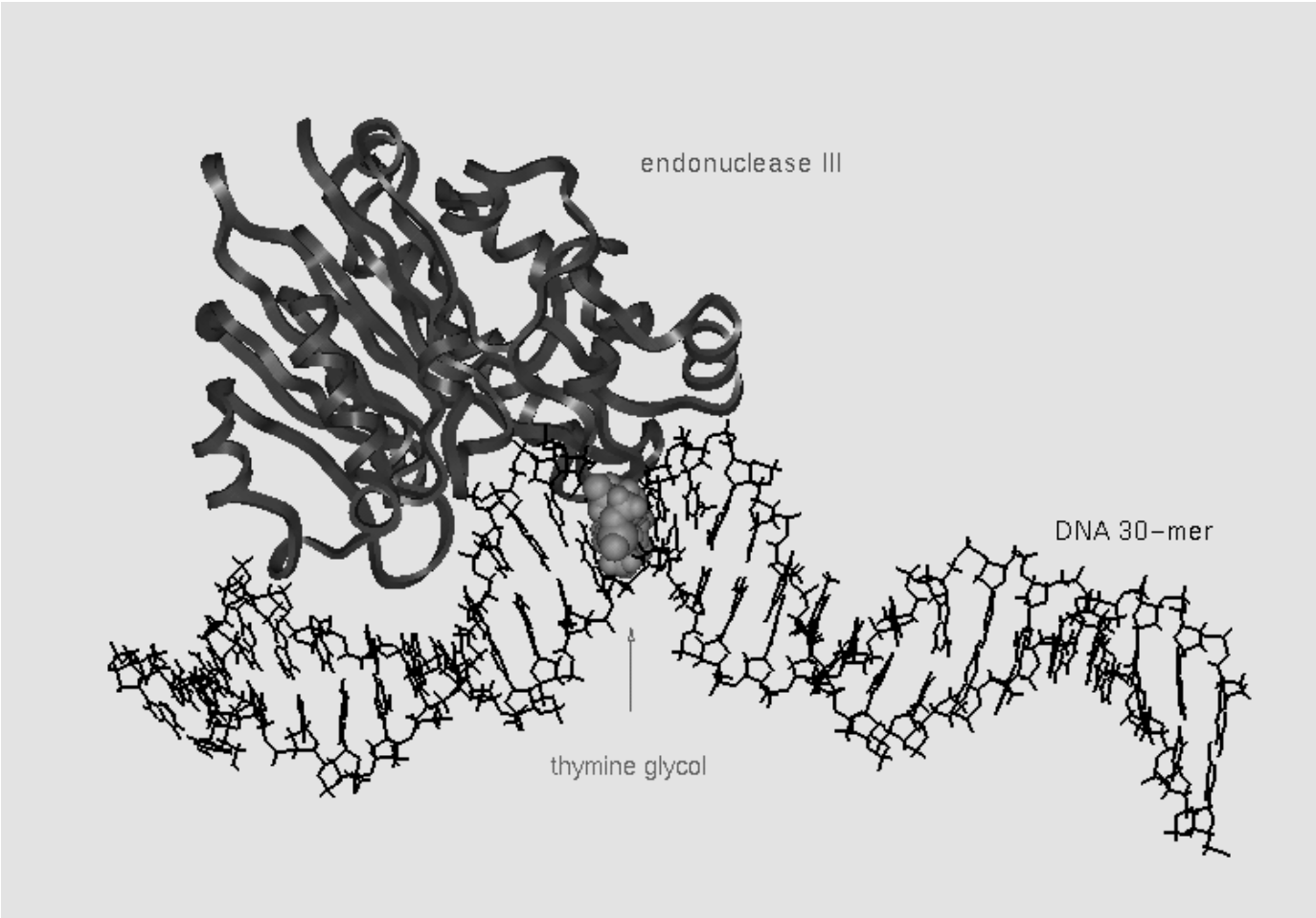


The closest atoms are as close as 6 Å, to minimize Van der Waals interactions. Glutamic acid is amino acid probably involved in scission of C5' and incision of C3' atom of TG (green) during repair process.



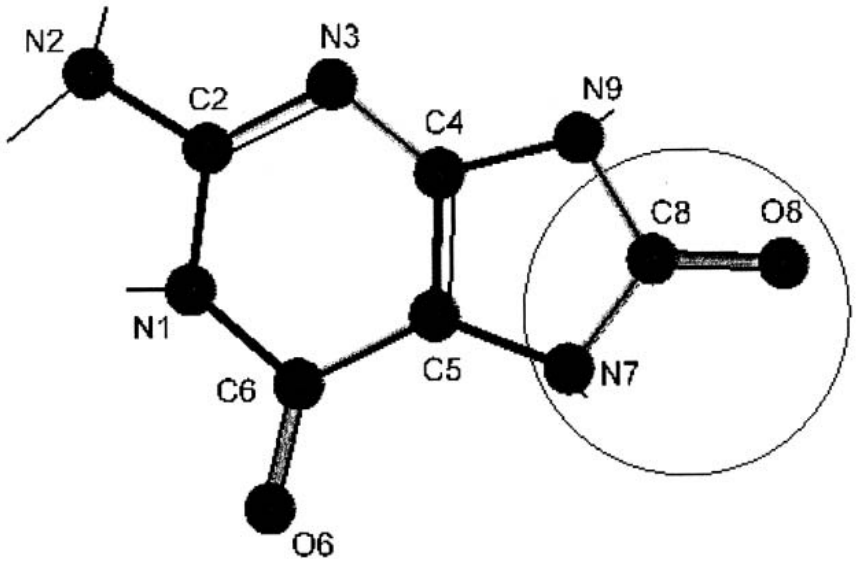
Snapshots of thymine glycol lesioned DNA. The molecule after 600 is bent and kinked at the TG site. TG is represented as yellow Connolly surface. The bending is expressed as the value of angle measured between phosphates of the respective nucleotides.

TG lesioned DNA 30-mer complexed with Endonuclease III

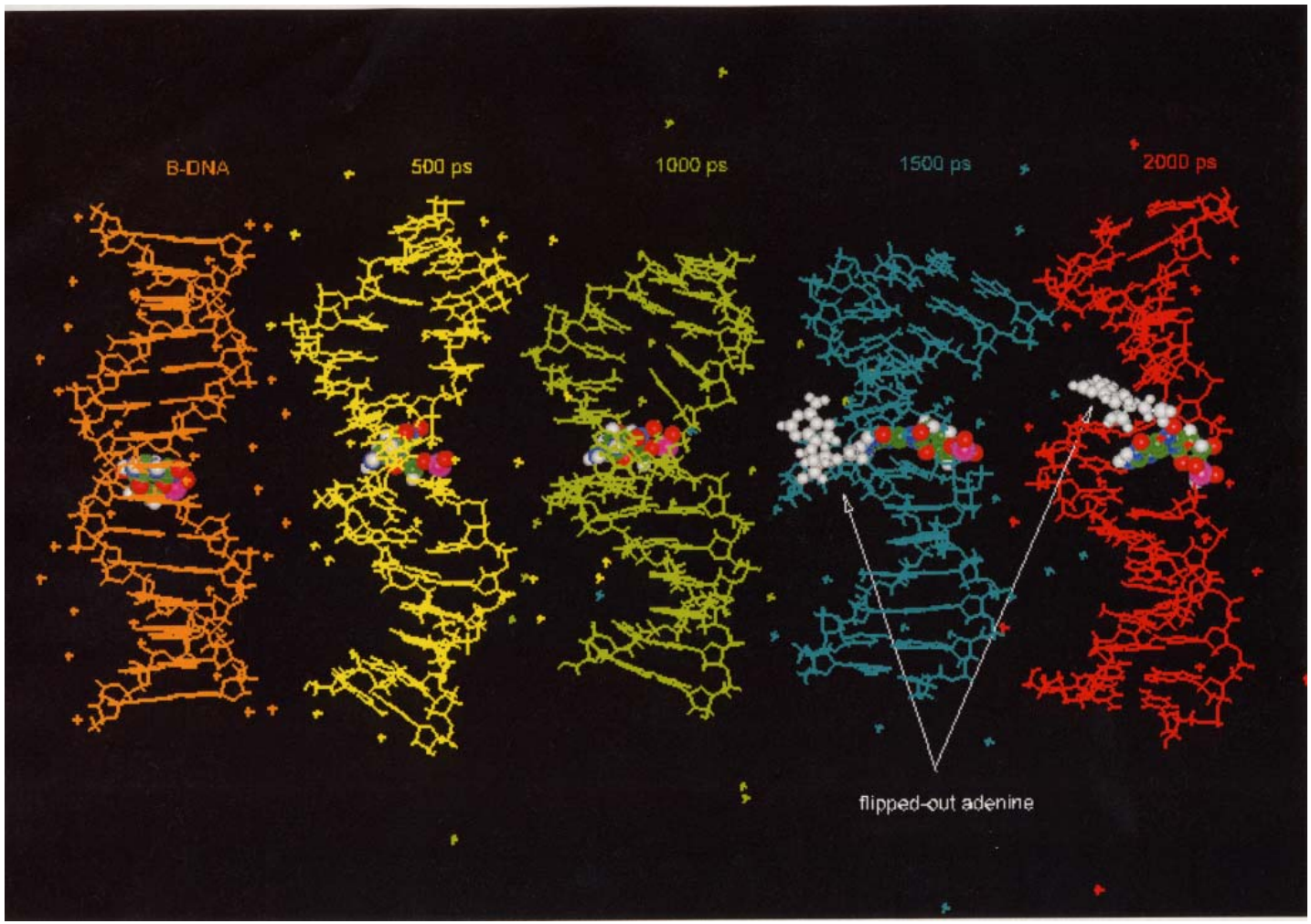


The DNA 30-mer and endonuclease III formed complex at 900 ps of MD. Stabile complex lasted until 2 ns when MD simulation was terminated.

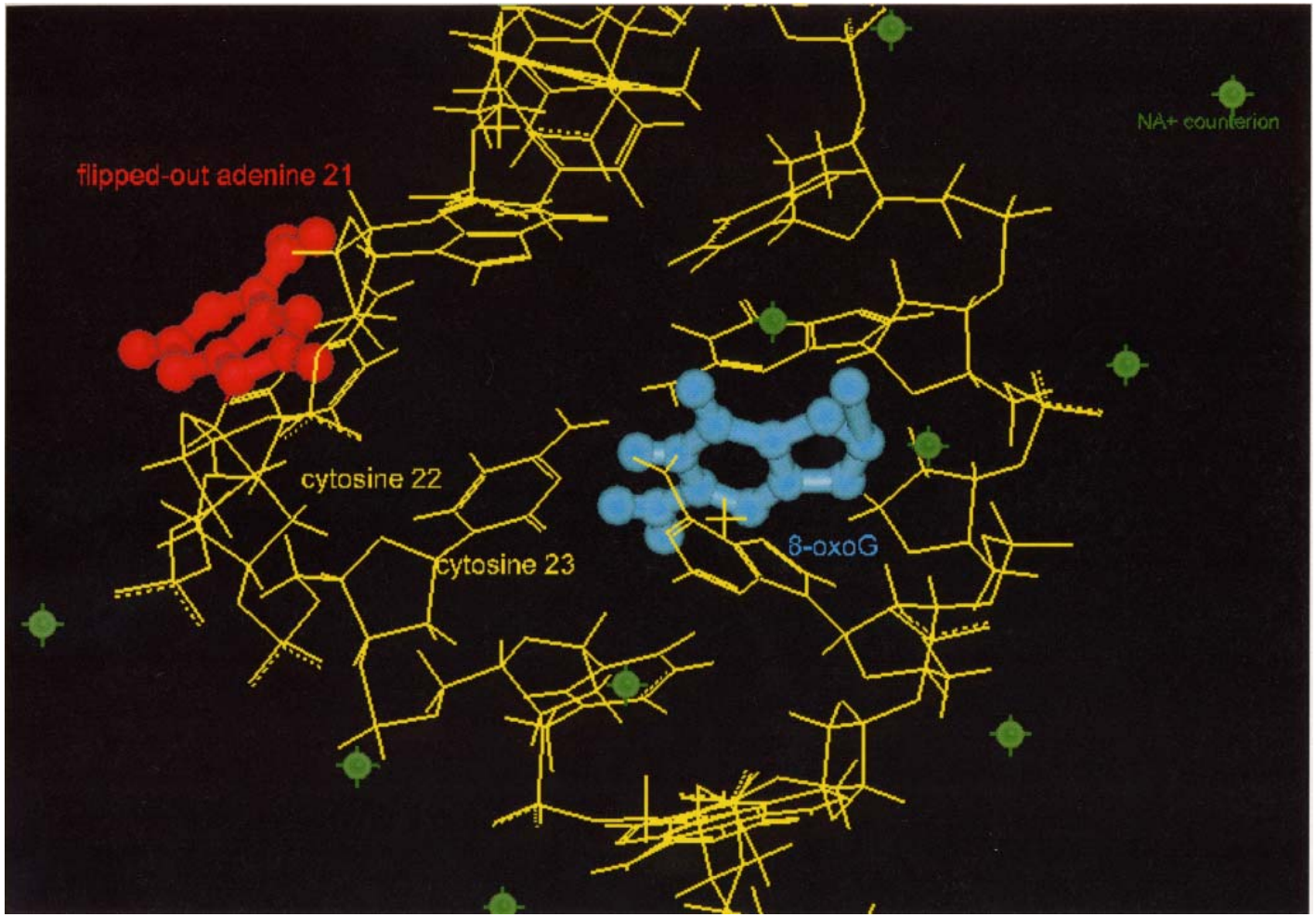
8-oxoguanine is considered to be one of the major endogenous mutagens contributing to spontaneous cells transformation vie miss pairing with adenine originating the G-C → T-A transversion mutations (the most common somatic mutation in human cancers).



The molecule of 8-oxoguanine formed as a 7,8-dihydro-8-oxoguanine by addition of oxygen atom on the C8 atom of guanine. (addition of oxygen (O8) at the C8, hybridization of the N7, transforming the double bond C8-N7 into single one)



Snapshots of DNA with 8-oxoguanine.
The flipped-out adenine 21 is seen at 1.5 and 2 ns.



Flipped-out adenine 21 on the complementary strand to the strand with 8-oxoguanine.
The distorted double helix indicates non-existence of hydrogen bonds, guanine 9 and cytosine 22 are dislocated from their intrahelical positions.

Electrostatic Energy in Biology and Chemistry

The role of electrostatic interactions:

- non bonded van der Waals and electrostatic interactions account for most interactions between molecules
- in general a positive charge surface in a solvent is screened by a negative charge surface
- molecules interact by molecular surface contact (the molecular surface is defined as the contact surface between the van der Waals envelope of the molecule and the probe solvent molecule)

The importance of electrostatic interactions for lesion recognition:

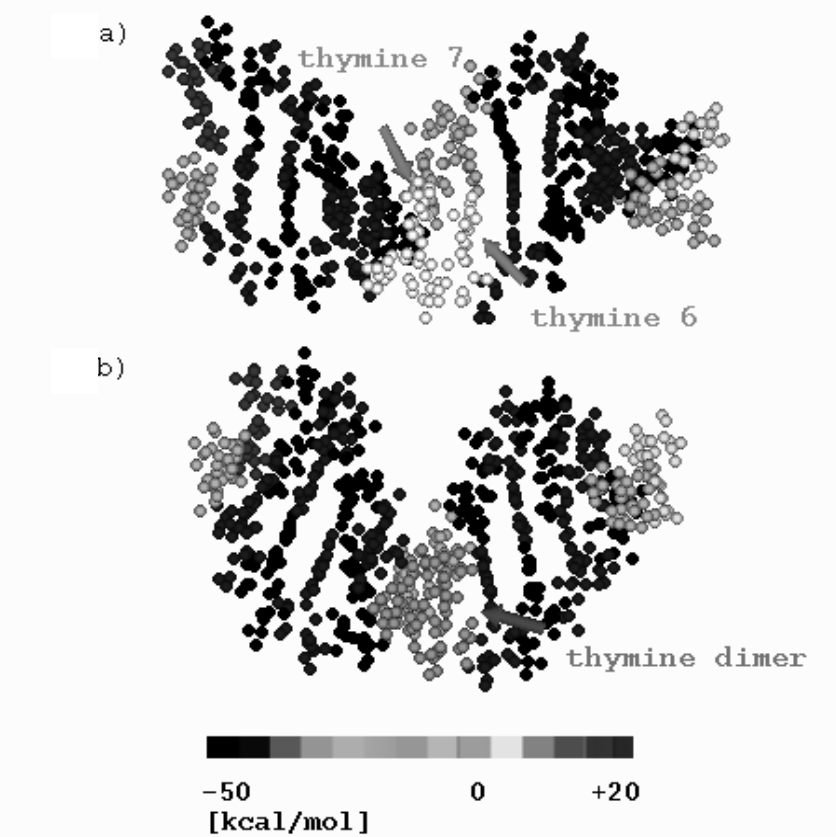
- ⇒ negative charge density generated by phosphodiester backbone causes equilibrated concentration of counterions in the vicinity of DNA surface
- ⇒ conformational properties, its stability and DNA regular interactions (e.g. with protein) depend on salt concentration, i.e. on the electrostatic properties of DNA
- ⇒ lesion may locally change electrostatic properties of DNA
- ⇒ arising specific interaction between protein and DNA may promote recognition

Electrostatic Energy of Selected Nucleotides

Native base	Lesion	Electrostatic Energy * [kcal/mol]
adenine		- 15
guanine		-37
	8-oxoguanine	-48
cytosine		-45
	cytosinyl radical	-
thymine		0
	thymine dimer	-10
	thymine glycol	-26

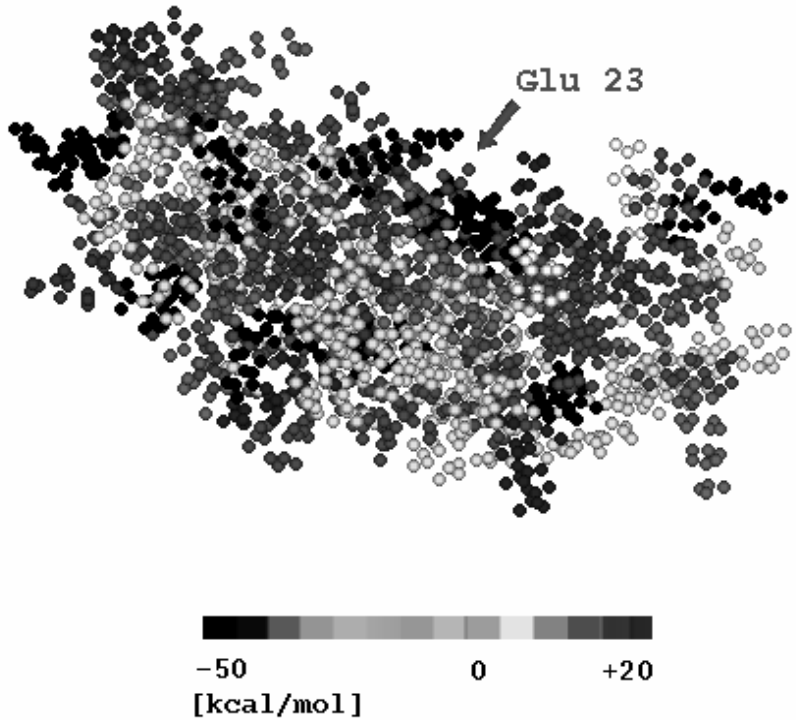
* value of electrostatic energy of the nucleotide with respective base

Electrostatic energy of nucleotides (thymine & thymine dimer)



a) native DNA, 0 kcal/mol
b) TD lesioned DNA, negative value of TD site 10 kcal/mol

Electrostatic energy of amino acids T4 endonuclease V



Most amino acids located at the surface show positive value of electrostatic energy (arginines are negative) Glutamin 23 – part of catalytic center has value of +10 kcal/mol.

Structural and Energetical Changes Observed in MD Simulations

1. Disrupted hydrogen bond network around the lesion:
   - cytosinyl radical
   - 8-oxoguanine
2. Sharp bending at the lesion
   - thymine dimer
   - thymine glycol
3. Flipped-out base
   - cytosinyl radical
   - 8-oxoguanine
4. Specific electrostatic interaction energy at the lesion
   - 8-oxoguanine
   - thymine dimer
   - thymine glycol